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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Titievsky *et al.*

Serial No.: **09/410,319**

Group Art Unit: **1655**

Filed: **October 1, 1999**

Examiner: **A. Chakrabarti**

For: **A NOVEL RET-INDEPENDENT SIGNALING PATHWAY FOR GDNF**

I, **Patrick J. Farley, Ph.D.**, Registration No. **42,524**, certify that this correspondence is being deposited with the U.S. Postal Service as First Class mail in an envelope addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

On DATE: Sept. 24, 2001

Patrick J. Farley, Esq.
Registration No: **42,524**

Assistant Commissioner
for Patents
Washington, D.C. 20231

Dear Sir:

In response to the Official Action dated May 24, 2001, please amend the application as follows. A petition for a one month extension of time is enclosed.

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In the Claims

Please substitute the following amended claims for those in the application as filed. A version to show changes made is enclosed.

Sub B1
A1

1. A method for identifying a compound that is an agonist of intracellular signaling effected by GPI-anchored receptors in nervous system cells comprising (i) incubating said nervous system cells having GPI-anchored receptors with a test compound and (ii) determining whether intracellular signaling has been effected in said cells, thereby identifying a compound that is an agonist of intracellular signaling effected by said GPI-anchored receptors.

Sub B2
A2

16. A method for identifying a compound that is an antagonist of intracellular signaling effected by GPI-anchored receptors in nervous system cells comprising (i) incubating said nervous system cells having GPI-anchored receptors with a test compound in the presence of a sufficient amount of an agonist of said intracellular signaling to effect intracellular signaling, and (iii) comparing the results to controls not incubated with said compound, thereby identifying a compound that is an antagonist of intracellular signaling effected by GPI-anchored receptors.

A3

30. A method for identifying a compound that is an agonist of GFRα1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells that express GFRα1 receptor, but not Ret receptor, with a test compound and (ii) determining whether intracellular signaling has been effected in said cells, thereby identifying a compound that is an agonist of GFRα1-dependent, Ret-independent intracellular signaling.

A4

39. A method for identifying a compound that is an antagonist of GFRα1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells that express GFRα1 receptor, but not Ret receptor, with a test compound in the presence of a sufficient amount of an agonist of

said intracellular signaling to effect intracellular signaling, and (iii) comparing the results to controls not incubated with said compound, thereby identifying a compound that is an antagonist of GFRα1-dependent, Ret-independent intracellular signaling.

49. A method for identifying a compound which is an agonist of GFRα1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells which express GFRα1 receptor, but not Ret receptor, with a test compound (ii) determining whether an increase in intracellular Ca^{2+} concentration is effected in said cells as compared to controls not incubated with said compound, thereby identifying a compound which is an agonist of GFRα1-dependent, Ret-independent intracellular signaling.

54. A method for identifying a compound which is an antagonist of GFRα1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells which express GFRα1 receptors, but not Ret receptors, with a compound to be tested in the presence of a sufficient amount of an agonist of GFRα1-dependent, Ret-independent intracellular signaling to cause an increase in intracellular Ca^{2+} concentration, and (ii) determining whether a decrease in intracellular Ca^{2+} concentration is effected, as compared with controls performed without said compound to be tested, thereby identifying a compound which is an antagonist of GFRα1-dependent, Ret-independent intracellular signaling.

59. A method for identifying a compound which is an agonist of GFRα1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells which express GFRα1, but not Ret, with the compound to be tested, (ii) preparing a cell lysate, (iii) immunoprecipitating the detergent insoluble fraction of the cell lysate with anti-GFRα1 antibodies to form an immunoprecipitate, and (iv) performing an assay for measuring kinase phosphorylation on said

a7 cont

immunoprecipitate, thereby identifying a compound which is an agonist of GFRα1-dependent, Ret-independent intracellular signaling.

a8

63. A method for identifying a compound which is an antagonist of the GFRα1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells which express GFRα1, but not Ret, with the compound to be tested in the presence of a sufficient amount of an agonist of said intracellular signaling to effect kinase phosphorylation ii) preparing a cell lysate, (iii) immunoprecipitating the detergent insoluble fraction of the cell lysate with anti-GFRα1 antibodies to form an immunoprecipitate, (iv) performing an assay for measuring kinase phosphorylation on said immunoprecipitate, and (v) comparing the results of said assay to those achieved in control experiments performed in the absence of said compound to be tested, thereby identifying a compound which is an antagonist of the GFRα1-dependent, Ret-independent intracellular signaling.

a9

68. A method for identifying a compound which is an agonist of GFRα1-dependent, Ret-independent intracellular signaling comprising (i) incubating cells which express GFRα1, but not Ret, with a compound to be tested, and (ii) determining whether activation of Src-type kinase is effected, as compared with controls not incubated with said compound, thereby identifying a compound which is an agonist of GFRα1-dependent, Ret-independent intracellular signaling.

a10

75. A method for identifying a compound which is an antagonist of the GFRα1-dependent, Ret-independent intracellular signaling pathway comprising (i) incubating cells which express GFRα1, but not Ret, with a compound to be tested in the presence of a sufficient amount of an agonist of said pathway to cause activation of Src-type kinase and (ii) determining whether said compound effects a decrease in Src-type kinase activation, as compared with controls not

incubated with said compound, thereby identifying a compound which is an antagonist of the GFRα1-dependent, Ret-independent intracellular signaling pathway.

83. A method for identifying a compound which is an agonist of intracellular signaling effected by GFRα receptors comprising (i) incubating lipid rafts prepared from cells having GFRα receptors with said compound and (ii) determining whether Src-type kinase is activated as compared to controls not incubated with said compound, thereby identifying a compound which is an agonist of intracellular signaling effected by GFRα receptors.

87. A method for identifying a compound which is an antagonist of intracellular signaling effected by GFRα receptors comprising (i) incubating lipid rafts prepared from cells having GFRα receptors with said compound in the presence of a sufficient amount of an agonist of the GFRα-dependent, Ret-independent intracellular signaling pathway to activate Src-type kinases and (ii) comparing the results to control experiments performed in the absence of said compound, thereby identifying a compound which is an antagonist of intracellular signaling effected by GFRα receptors.

Remarks

The claims in the case are 1-115. Applicants elected claims 1-91 in the response to the Requirement for Restriction. Thus, claims 1-91 are currently under examination.

Applicants have amended claims 1, 16, 30, 39, 49, 54, 59, 63, 68, 75, 83, and 87, as helpfully suggested by te Examiner, to include in the last step of each of the claims a phrase that states the accomplishments of the goals for the method which were stated in the methods' preambles. We earnestly submit that claims 1, 16, 30, 39, 49, 54, 59, 63, 68, 75, 83, and 87 are in proper form for allowance.

The Applicants have discovered methods of identifying agonists and antagonists of GPI-linked receptors in nervous system cells that affect intracellular signaling. Specifically, glial cell-derived neurotrophic factor (GDNF), a TGF-β family member which is believed to have possible importance in Alzheimer's Disease and Parkinson's Disease, has been thought to act through a multi-component system involving other cellular proteins including GFRα1, a GPI-linked receptor of GDNF, and c-Ret, a transmembrane protein receptor kinase. There had been scant evidence that a GPI-linked receptor alone could affect intracellular signaling. The inventors of the present application have discovered a method of identifying agonists and antagonists of GPI-linked receptors using the GDNF system in which the intracellular signaling is independent of transmembrane proteins such as Ret.

The claims Are Novel Over Jefferies

Turning to the merits of the invention, claims 1 and 16 are patentable over U.S. Patent No. 5,981,194 to Jefferies *et al.* ("Jefferies"). Jefferies discloses at Col. 8, lines 30-48 (cited by the Examiner) a method of identifying an agonist or antagonist of p97, a GPI-anchored protein, by reacting a substance suspected of being an agonist or antagonist of p97 with a cell expressing p97. However, Jefferies methods determines the amount of p97 expressed by the cell in relation to the amount of p97 expressed by control cells. Jefferies' method does not determine whether intracellular signaling has been effected. In another method described by Jefferies in this passage, p97-mediated iron uptake is measured, not intracellular signaling. The Examiner states that iron uptake in the cell inherently causes intracellular signaling, yet provides no reference for such an assertion. Moreover,

at best, iron uptake is an upstream event of any potential intracellular signaling. Jefferies does not teach or suggest that iron uptake correlates with intracellular signaling such that measurement of iron uptake could be used by one of ordinary skill in the art as an index of intracellular signaling. Thus, the Examiner fails to provide a *prima facie* case that Jefferies inherently anticipates the claims, which are drawn to determining agonists and antagonists of intracellular signaling, not iron uptake itself. Applicants submit that claims 1 and 16 are patentable over Jefferies.

The Claims Are Nonobvious Over the Cited Art

The Examiner has rejected claims 1-6, 16-21, 30, 32, 39, 40 and 42 under 35 U.S.C. §103(a) over Ibanez *et al.* (WO 97/18240)("Ibanez") in view of Jefferies, further in view of Cacalano et al. (*Neuron* (1998) 21:53-62)("Cacalano").

Independent Claim 1 recites a method for identifying a compound that is an agonist of a GPI-anchored receptor in a nervous system cell. The Examiner cites Ibanez and alleges that Ibanez teaches intracellular signaling of GDNF via c-Ret. Note that c-Ret is a transmembrane protein, not a GPI-linked protein.

The hypothetical combination of Ibanez with Jefferies would not achieve the Applicants invention in that Jefferies describes a method of identifying agonists and antagonists of p97, a GPI-linked protein that is suspected of modulating iron transport in a cell, not affecting intracellular signaling. There is no motivation to combine the two references as one Ibanez is directed to GDNF signaling through a transmembrane receptor and Jefferies is directed to assays for protein expression or iron uptake resulting from compounds interacting with an unrelated GPI-linked receptor. Moreover, substituting the detection mechanism in Jefferies (measurement of p97 expression or iron

uptake in the absence of transferrin) in the system described by Ibanez would neither be directed to GPI-linked GDNF receptors, nor measure intracellular signaling. Thus, there is no reasonable expectation of success in achieving the instantly claimed invention.

The Examiner states that the rejected claims are obvious over Ibanez in view of Jeffries, further in view of Cacalano. Calcalano is cited for teaching a GPI-linked receptor for GDNF, termed GFRα1. The Examiner appears to argue that it would have been obvious to one of ordinary skill in the art to find agonists or antagonists of GFRα1 (a GPI-linked protein) by using the method of Ibanez (identifying agonists/antagonists of c-Ret, a transmembrane protein) and determining whether there is increased expression of protein or iron uptake (in view of Jefferies).

We invite the Examiner's attention to the first paragraph of the discussion in Cacalano wherein it is stated that "the receptors for the GDNF protein family are composed of two subunits: a GPI-linked ligand binding protein that belongs to the GFRα family and a signaling component that is represented by the transmembrane tyrosine kinase Ret." It is clear that Cacalano teaches that intracellular signaling occurs through Ret and not via a GPI-linked protein. Thus, there is no motivation to combine the teachings of Cacalano with Ibanez in view of Jefferies to achieve a method of identifying agonists or antagonists of a GPI-linked protein in nervous system cells by examining intracellular signaling via the GPI-linked protein. The claimed invention is for GPI-linked protein directed intracellular signaling (note that independent claim 39 even specifically references "ret-independent" signaling).

We respectfully submit that the claims, as amended are patentable over the hypothetical combination of Ibanez, Jefferies and Cacalano.

The Examiner has rejected claims 1-10, 15-24, 29-33, 38-40, 42, 43, and 48-58 under 35 U.S.C. §103(a) over Ibanez in view of Jefferies, further in view of Cacalano, further in view of Shen *et al.* (*J. Immunol.* (1994) 152:3017-3023)("Shen").

Shen is cited for methods of measuring intracellular signaling. However, as set forth above, the hypothetical combination of Ibanez, Jefferies and Cacalano fails to achieve the basic steps of the Applicants' invention. Shen adds nothing to make up for the fundamental deficiency of failing to provide sufficient motivation to combine the references in a manner that could possibly achieve the claimed invention.

The Examiner has rejected claims 1-10, 12-13, 15-24, 26-27, 29-33, 35-36, 38-40, 42, 43, 45-46, 48-58, 68-70, 75-77, 79-85, 87-89, and 91 under 35 U.S.C. §103(a) over Ibanez in view of Jefferies, further in view of Cacalano, further in view of Shen, further in view of Dikic *et al.* (*Nature* (1996) 383:547-549).

Dikic is cited for a method of measuring intracellular signaling via a Src-type kinase by activation of MAPK. However, as set forth above, the hypothetical combination of Ibanez, Jefferies and Cacalano fails to achieve the basic steps of the Applicants' invention. Shen and Dikic add nothing to make up for the fundamental deficiency of failing to provide sufficient motivation to combine the references in a manner that could possibly achieve the claimed invention.

The Examiner has rejected claims 1-10, 12-24, 26-33, 35-40, 42, 43, 45-58, 68-71, and 75-91 under 35 U.S.C. §103(a) over Ibanez in view of Jefferies, further in view of Cacalano, further in view of Shen, further in view of Dikic *et al.* (*Nature* (1996) 383:547-549), further in view of Finkbeiner *et al.* (*Neuron* (1997) 19:1031-1047).

Finkbeiner is cited for a method of measuring intracellular signaling via a Src-type kinase by activation of CREB. However, as set forth above, the hypothetical combination of Ibanez, Jefferies and Cacalano fails to achieve the basic steps of the Applicants' invention. Shen, Dikic and Finkbeiner add nothing to make up for the fundamental deficiency of failing to provide sufficient motivation to combine the references in a manner that could possibly achieve the claimed invention.

The Examiner has rejected claims 1-91 under 35 U.S.C. §103(a) over Ibanez in view of Jefferies, further in view of Cacalano, further in view of Shen, further in view of Dikic *et al.* (*Nature* (1996) 383:547-549), further in view of Finkbeiner *et al.* (*Neuron* (1997) 19:1031-1047), further in view of Chalazonitis *et al.* (*Developmental Biol.* (1998) 204:385-406).

Chalazonitis is cited for the use of an anti-GFRα1 antibody. However, as set forth above, the hypothetical combination of Ibanez, Jefferies and Cacalano fails to achieve the basic steps of the Applicants' invention. Shen, Dikic, Finkbeiner and Chalazonitis add nothing to make up for the fundamental deficiency of failing to provide sufficient motivation to combine the references in a manner that could possibly achieve the claimed invention.

We earnestly submit that the claims, as amended, are in proper form for allowance and are patentable over the cited art, either alone or in combination. We respectfully urge reconsideration of the claims and an early indication of allowance.

Respectfully submitted,

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